Pretreatment serum interferon gamma inducible protein-10 as biomarker of fibrosis and predictor of virological response in genotype 4 hepatitis C virus infection

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Abstract

Objective : **Assess the value of baseline interferon-γ-inducible protein-10 (IP-10) levels as a noninvasive maker of liver fibrosis and as a predictor of response to interferon therapy in HCV genotype 4 infected patients.**

Methods : **Eighty-four HCV genotype 4 infected patients were enrolled in this study. Degrees of liver fibrosis were determined and baseline IP-10 was measured in serum samples collected prior to initiation of treatment using the enzyme-linked immunosorbent assay. Patients were followed up for 1.5 year to assess their response to antiviral therapy.**

Results : **The baseline IP-10 levels were significantly correlated with the degree of fibrosis and had the ability to differentiate between patients with mild, moderate and advanced stages of fibrosis (F0-1 : 95.24 ± 33.08 pg/ml, n = 25 ; F2 : 158.70 ± 52.74 pg/ml, n = 37 ; F3-4 : 357.45 ± 162.18 pg/ml, n = 22 ; P < 0.001). Baseline IP-10 levels were significantly lower in patients achieved Early virological response (responders 134.80 ± 60.47 pg/ml, n = 60 ; non-responders 334.54 ± 168.94 pg/ml, n = 24, P < 0.001). Also baseline IP-10 levels were significantly lower in patients who became HCV RNA negative at 24 weeks of therapy (179.52 ± 130.03 pg/ml, n = 78) than non-responders (352.33 ± 132.58 pg/ml, n = 6, P = 0.002). SVR was achieved in 58/68 (85.3%) patients while 10 patients were relapsed. Baseline IP-10 levels differs significantly between patients who achieved SVR at week 24 post therapy and relapsed patients (IP10 level : SVR, 173.52 ± 125.20 pg/ml, n = 58 ; Relapsed, 216.20 ± 67.72 pg/ml, n = 10, P = 0.021).**

Conclusion : **Baseline IP-10 level independently predicts EVR, response at week 24 during therapy and SVR. It also differentiates patients with mild fibrosis from those with moderate and advanced fibrosis.** (Acta gastroenterol. belg., **2014**, 77, **401-407**).

Key words : IP-10, HCV, fibrosis, virologic response.

Abbreviation : IP-10, HCV, EVR, SVR.

Introduction

Hepatitis C virus (HCV) infects up to 180 million people worldwide (1) and is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (2). The current treatment, based on the combination of peginterferon alpha and ribavirin, leads to a sustained virological response (SVR) in 40-50% in patients with HCV genotype 1 and in 80% of those with genotype 2 or 3 (3).

Response rates to PEG-IFN and ribavirin are associated with both viral and host factors. Pretreatment predictors of nonresponse include genotype 1 infection, high viral load (> 800,000 IU/mL), advanced fibrosis or cirrhosis, high body mass index, age > 40 years, and African American race (4). Currently, on-treatment predictors of response to PEG-IFN and ribavirin include viral kinetics at weeks 4 and 12. Patients who do not attain an early virological response have only a 1%-3% chance of viral clearance, and therapy is usually halted (4,5). Conversely, 87% of patients who achieve a rapid virological response achieve SVR (6).

Although viral kinetics have proven useful, better predictors of SVR and nonresponse would be helpful to identify patients with the best chance of response before the initiation of combination antiviral therapy. Chemokines and cytokines are attractive as potential markers for treatment outcome because they are regulators of immunity and inflammation in HCV infection. Many are modulated by exogenous interferon and play critical roles in viral clearance (7).

Chemokines are critical regulators of immunity and inflammation in all phases of HCV infection. They function within cytokine cascades that regulate the immune response to the virus. However in chronic infection their persistent expression can drive chronic inflammation in the absence of effective anti-viral immunity leading to liver injury and cirrhosis (8).

Chemokines are involved in fibrosis both indirectly by recruiting inflammatory cells that drive fibrogenesis and also by direct effects on hepatic stellate cells which play a central role in fibrogenesis following their transition to myofibroblasts (9).

Liver biopsy currently remains the reference standard for assessment of hepatic histology. The limitations of the procedure, including its repeatability and reproducibility, have prompted a search for noninvasive markers of hepatic fibrosis. Although significant advances have been achieved, none of the currently available indices has sufficient accuracy to replace liver biopsy in the assessment of hepatic histology of chronic HCV infection. The

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primary limitation of these indices is an inability to identify patients with intermediate stages of fibrosis. Given the limitations of currently available noninvasive indices, novel biomarkers with improved discriminatory ability are required (10).

CXCL10 (IP-10) is part of a family of α-chemokines that bind CXCR3, which also includes CXCL9 and CXCL11. While all three ligands are induced by IFN and bind the same receptor, there now exist substantial data to support their unique roles in disease pathogenesis. This is clearly evident in chronic HCV, where elevated levels of all three have been demonstrated (11).

The aim of this study was to address whether peripheral IP-10 levels might have prognostic usefulness as biomarkers of hepatic fibrosis and predictor of virological response to interferon and ribavirin therapy in HCV genotype 4 infected patients.

Methods

Study Subjects

This study was approved by the ethical committee of the faculty of medicine, Menoufia University. All patients provided signed informed consent to provide a blood sample and to review the medical record for research purposes. Patients were eligible if they had new untreated chronic genotype 4 HCV infection and those who had any cause of liver disease other than HCV were excluded from participation in the study. FibroScan was used for assessment of the liver fibrosis stage before initiation of antiviral therapy. Serum samples were collected from 84 HCV genotype 4 infected patients before initiation of antiviral therapy (interferon + ribavirin) and stored at -80 until analysis of IP-10. Patients were followed up for 1.5 year to assess their response to antiviral therapy. Viral loads were measured before treatment, at weeks 12, 24 during treatment and at week 24 after stopping therapy to evaluate the virologic response. A rapid virological response (RVR) was defined as undetectable HCV RNA in serum at week 4 of therapy. Early virological response (EVR) was defined as serum HCV RNA negativity or any > 2 log10 decline in HCV RNA levels at week 12 of therapy compared with baseline. Patients with sustained virological response (SVR) were those with undetectable HCV RNA in serum 24 weeks after stopping therapy. Patients who had a < 2 log10 drop in viral load at week 12 as compared to baseline and those whose HCV RNA was still detectable at week 24 were considered nonresponders (according to international guidelines) (12).

Measurement of plasma chemokine levels

IP-10 was measured in serum samples collected at baseline, prior to initiation of treatment, using the commercially available enzyme-linked immunosorbent assay kits (Invitrogen, USA) according to the manufacturer's instructions.

Table 1. — **Baseline patients' characteristics**

	All patients (n = 84)
Age	40.88 ± 5.15
Gender Male Female	 66 (78.6) 18 (21.4)
Hb (g/dl)	13.8 ± 1.6
Platelets (× 10^3/μL)	210.6 ±53.7
WBCs (× 10^3/μL)	5.9 ± 1.9
AST(IU/L)	44.6 ± 20.9
ALT(IU/L)	49.9 ± 26.0
ALT/AST	1.12 ± 0.25
AST/ALT	0.94 ± 0.21
AST/Platelets (APRI test)	0.56 ± 0.30
HCV RNA (IU/mL)	1334146 ± 2822087
Fibrosis F0 F1 F2 F3 F4	 3 (3.6) 22 (26.2) 37 (44) 19 (22.6) 3 (3.6)

ALT, alanine aminotransferase ; AST, aspartate aminotransferase.
Data are expressed as mean ± S.D. or number (%).

Results

Patients Included in the Study

Eighty four HCV genotype 4 patients were included in this analysis. Baseline patient characteristics were shown in table 1. Viral load at week 4 was available for 11 patients only, 4 of them achieved RVR. Early virological response at week 12 occurred in 60/84 (71.4%) patients. Non response to antiviral therapy at week 24 was present in 6/84 (7%) patients. The definitions of responder and nonresponder are provided in Patients and Methods. SVR was achieved in 58/68 (85.3%) patients while 10 patients were relapsed and the remaining 16 patients were lost follow up at week 24 after stopping therapy.

IP-10 levels and fibrosis

The IP-10 level differed significantly among patients with different stages of fibrosis (F0-1 : 95.24 ± 33.08 pg/ml, n = 25 ; F2 : 158.70 ± 52.74 pg/ml, n = 37 ; F3-4 : 357.45 ± 162.18 pg/ml, n = 22 ; P < 0.001 ; figure 1 and table 2). Comparison between fibrosis (stage 0-1, n = 25 and stage 2-4, n = 59) using other parameters like ALT levels, ALT/ AST ratio and AST/ platelet ratio (APRI test) showed that the APRI test was the only one significantly associated (P value < 0.01). Comparison between IP-10 and APRI test using ROC curve to differentiate between patients with mild fibrosis grade 0-1 and those with moderate/severe fibrosis grade 2-4 revealed that IP-10 had the best discriminatory ability (AUROC ± SE = 0.91 ± 0.03 ; 95% CI = 0.85-0.97 ; P value < 0.001) than APRI test (AUROC ± SE = 0.68 ± 0.06 ;

Table 2. — **Comparison between different stages of fibrosis and responders and non responders to interferon therapy as regards IP-10 levels**

	IP-10 (pg/ml) X ± SD	Test of significance	P value
Fibrosis F0-1 (25) F2 (37) F3-4 (22)	 95.24 ± 33.08 158.70 ± 52.74 357.45 ± 162.18	56.80	< 0.001
Fibrosis F0-1 F2-3-4	 95.24 ± 33.08 232.81 ± 143.69	5.95	< 0.001
Early virological response Responder (60) Non responder (24)	 134.80 ± 60.47 334.54 ± 168.94	6.05	< 0.001
24 weeks virological response Responder (78) Non responder (6)	 179.52 ± 130.03 352.33 ± 132.58	3.10	0.002
SVR Responder (58) Non responder (10)	 173.52 ± 125.20 216.20 ± 67.72	2.31	0.021

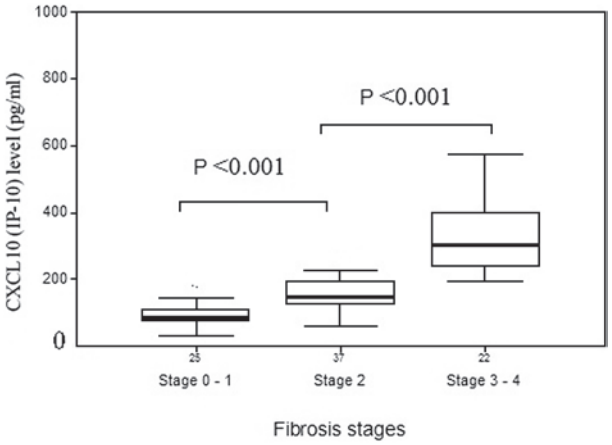

Fig. 1. — IP-10 (CXCL10) levels in different fibrosis stages. Significantly higher levels of IP-10 were found in advanced stage of fibrosis (stage 3-4) compared with mild (stage 0-1) and moderate stage (stage 2).

95% CI = 0.56-0.80 ; P value < 0.01). Using a cutoff value of 107.5 pg/ml for IP-10, we obtained a sensitivity of 91.5%, a specificity of 72%, a positive predictive value of 88.5%, and a negative predictive value of 78.3%. When we evaluated the APRI test with use of a cutoff value of 0.4185, we obtained a sensitivity of 71.2%, a specificity of 56%, a positive predictive value of 79.2%, and a negative predictive value of 45.2%. In comparison with the APRI, the IP-10 level has higher sensitivity and specificity. Combined IP-10 and APRI test have a sensitivity of 94.9%, a specificity of 72%, a positive predictive value of 75.7% and a negative predictive value of 70%. IP-10 also had the ability to differentiate between patients with mild/ moderate fibrosis grade 0-2 from those with severe fibrosis grade 3-4 (AUROC ± SE = 0.97 ± 0.02 ; 95% CI = 0.97-1.0 ; P value < 0.001). Using a cutoff value of 190 pg/ml for IP-10, we obtained a sensitivity of 100%, a specificity of 82.3%, a positive predictive value of 66.7%, and a negative predictive value of 100%.

Baseline Serum IP-10 Measurement and Treatment Response

RVR was observed in 4/11 (36.4%) patients. Baseline IP-10 levels were not significantly associated with RVR (responders, 108.75 ± 77.82 pg/ml, n = 4 ; nonresponders, 276.42 ± 262.04 pg/ml, n = 7, P value = 0.185).

EVR was observed in 60 / 84 (71.4%) patients. Baseline IP-10 levels were significantly lower in responders (134.80 ± 60.47 pg/ml, n = 60) versus nonresponders (334.54 ± 168.94 pg/ml, n = 24, P value < 0.001, table 2). To assess the potential predictive value of IP-10 measurements for EVR, we used ROC curve analysis to differentiate between responders and non-responders. IP-10 levels had a good discriminatory ability (AUROC ± SE = 0.92 ± 0.03, 95% CI = 0.87-0.98, P value < 0.001). Using a cutoff value of 190 pg/ml, we obtained a sensitivity of 83.3%, a specificity of 78.3%, a positive predictive value of 86.9%, and a negative predictive value of 92.2%. Association between other parameters and EVR were not significant except for age and the degree of fibrosis (P value < 0.001) table 3.

Assessment of response to treatment at week 24 during therapy showed that 6/84 (7%) patients were non responder (treatment failure). Baseline IP-10 levels differs significantly between responders (179.52 ± 130.03 pg/ml, n = 78) and non-responders (352.33 ± 32.58 pg/ml, n = 6, P value < 0.002) at week 24 during therapy (table 2). ROC curve analysis to dif-

Table 3. — **Comparison between responders and non-responders at week 12 during therapy using different parameters**

	Early virological response		Mann Whitney U test	P value
	Responders N = 60	Non responders N = 24		
Age Range	39.4 ± 4.84 28 – 50	44.58 ± 3.93 38 – 50	t- test 4.66	< 0.001
Sex Male Female	45 (75%) 15 (25%)	21 (87.5%) 3 (12.5%)	X^2 1.59	0.21
AST (IU/L)	42.32 ± 18.36	51.54 ± 25.51	1.88	0.06
ALT (IU/L)	47.15 ± 21.47	56.97 ± 34.48	0.79	0.43
ALT/AST	1.13 ± 0.25	1.08 ± 0.25	0.79	0.43
AST/Platelets	0.65 ± 0.38	0.53 ± 0.25	1.01	0.31
HCV RNA (IU/mL)	1469557 ± 3229952.4	995617.1 ± 1345735.51	0.12	0.90
Fibrosis F0 F1 F2 F3 F4	 3 (5.0) 22 (36.7) 32 (53.3) 3 (5.0) 0 (0.0)	 0 (0.0) 0 (0.0) 5 (66.7) 16 (20.8) 3 (12.5)	50.43	< 0.001
Fibrosis F0-1 F2 F3-4	 25 (41.7) 32 (53.3) 3 (5.0)	 0 (0.0) 5 (20.8) 19 (79.2)	50.11	< 0.001

Data are expressed as mean ± S.D. or number (%).

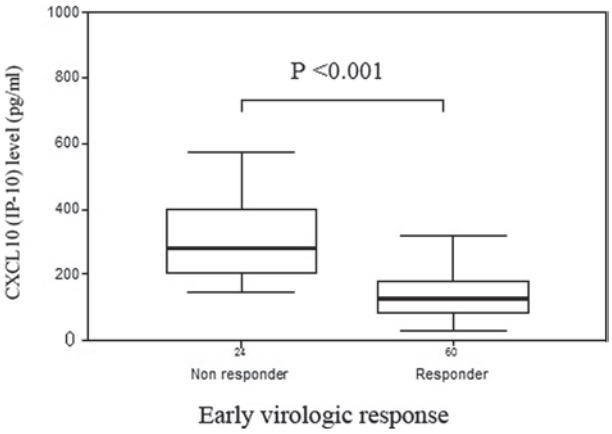

Fig. 2. — IP-10 (CXCL10) levels at week 12 of therapy. Significantly lower levels of IP-10 were found in patients achieved EVR than those who were non responding.

ferentiate between responders and non-responders at 24 weeks of therapy by IP-10 level revealed that : AUROC ± SE = 0.88 ± 0.05, 95% CI = 0.79-0.98, P value < 0.002. At cutoff value of 190 pg/ml, we obtained a sensitivity of 100%, a specificity of 65.4%, a positive predictive value of 18.2%, and a negative predictive value of 100%. Association of other parameters with response to treatment at week 24 during therapy were not significant except for age and the degree of fibrosis (P value < 0.001) table 4.

SVR was achieved in 58/68 (85.3%) patients while 10 patients were relapsed. Baseline IP-10 levels differ significantly between patients who achieved SVR at week 24 post therapy and relapsed patients (IP10 level : SVR, 173.52 ± 125.20 pg/ml, n = 58 ; Relapsed, 216.20 ± 67.72 pg/ml, n = 10, P = 0.021) figure 4, table 2. ROC curve analysis to differentiate between SVR and relapsed cases by IP-10 level revealed that : AUROC ± SE = 0.73 ± 0.06, 95% CI = (0.60- 0.86), P value = 0.021. At cutoff value of 190 pg/ml, we obtained a sensitivity of 60%, a specificity of 69%, a positive predictive value of 25%, and a negative predictive value of 91%.

Association between other parameters and SVR were not significant except for viral load (P value < 0.001) and the degree of fibrosis (P value = 0.024) table 5. ROC curve analysis for SVR using viral load revealed : AUROC ± SE = 0.89 ± 0.04, 95% CI = (0.80-0.98), P value < 0.001. At viral load cutoff value of 61584 IU/ml, we obtained a sensitivity of 100%, a specificity of 29%, a positive predictive value of 19%, and a negative predictive value of 100%. Combined IP10 and viral load have a sensitivity of 80%, a specificity of 19%, a positive predictive value of 15% and a negative predictive value of 85%.

Discussion

Hepatic fibrogenesis is a dynamic process that may be initiated with viral infection of hepatocytes and that is subsequently amplified by intrahepatic inflammation that develops as a result of the infection. CXCR3-associated chemokines, through their chemoattractive properties, likely play a pivotal role in the initiation and perpetuation

Table 4. — **Comparison between responders and non-responders at week 24 during therapy using different parameters**

	24 weeks virological response		Mann Whitney U test	P value
	Responders N = 78	Non responders N = 6		
Age Range	40.55 ± 5.13 28 – 50	45.17 ± 3.25 42 – 50	t- test 4.66	< 0.001
Sex Male Female	 62 (79.5%) 16 (20.5%)	 4 (66.7%) 2 (33.3%)	*Fisher's Exact test* 0.54	0.60
AST	43.90 ± 20.40	58.50 ± 24.76	1.51	0.13
ALT	48.67 ± 25.82	66.0 ± 24.99	1.72	0.08
ALT/AST	1.11 ± 0.25	1.17 ± 0.30	0.12	0.90
AST/Platelets	0.76 ± 0.33	0.55 ± 0.29	1.61	0.11
HCV RNA (IU/mL)	1356006 ± 2903103.3	10449968 ± 1519748.91	0.61	0.54
Fibrosis F0 F1 F2 F3 F4	 3 (3.8) 22 (28.2) 37 (47.4) 15 (19.2) 1 (1.3)	 0 (0.0) 0 (0.0) 0 (0.0) 4 (66.7) 2 (33.3)	26.34	< 0.001
Fibrosis F0-1 F2 F3-4	 25 (32.1) 37 (47.4) 16 (20.5)	 0 (0.0) 0 (0.0) 6 (100)	18.21	< 0.001

Data are expressed as mean ± S.D. or number (%).

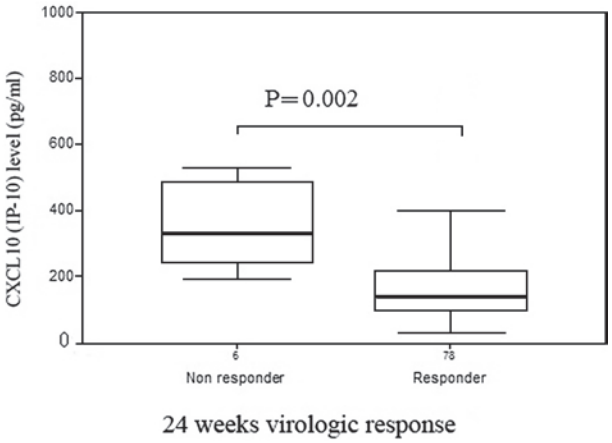

Fig. 3. — IP-10 (CXCL10) levels at week 24 of therapy. Significantly lower levels of IP-10 were found in patients achieved HCV RNA negativity than those who were non responding.

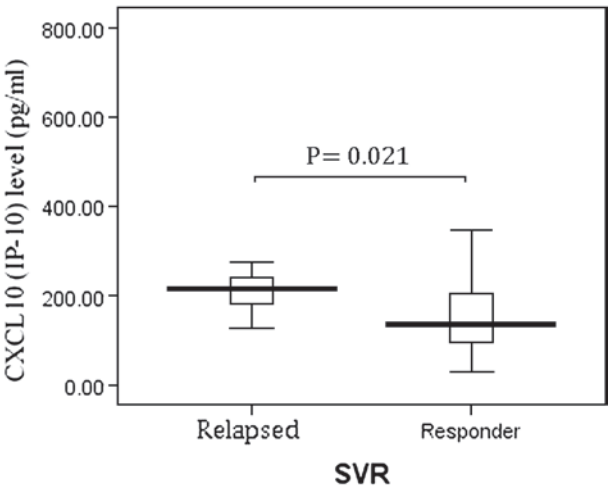

Fig. 4. — IP-10 (CXCL10) levels at week 24 post therapy. Significantly lower levels of IP-10 were found in patients achieved SVR than relapsed patients.

of the inflammatory cascade that is a disease-defining characteristic in chronic HCV infection. CXCL9-CXCL11 have been shown to be overexpressed in the liver and peripheral blood of patients who have chronic HCV infection, compared with uninfected individuals (13-18). In addition, lymphocytes expressing CXCR3 have been observed to predominate in the hepatic parenchyma of patients with hepatitis C (15,19,20). A significant association between the intrahepatic mRNA levels of all 3 CXCR3-associated chemokines and necroinflammatory grade as well as fibrosis stage was also reported (13). These findings prompted us to investigate the association between peripheral levels of CXCL10 (IP-10) and hepatic fibrosis. We observed that the levels of IP-10 were significantly associated with fibrosis stage. We also found that IP-10 had a good discriminatory ability in differentiating patients with mild fibrosis from those with moderate /advanced fibrosis and also differentiating mild/moderate fibrosis from those with advanced fibrosis. These results were similar to those reported by

Table 5. — **Comparison between responders and non-responders at week 24 post therapy using different parameters**

	24 weeks post therapy virological response		Mann Whitney U test	P value
	Responders N = 58	Non responders N = 10		
Age	40.15 ± 4.70	42.30 ± 7.46	1.08	0.277
Sex Male Female	 45 (77.6%) 13 (22.4%)	 9 (90%) 1 (10%)	*Fisher's Exact test* 0.80	0.674
AST	44.39 ± 22.26	43.30 ± 11.19	0.79	0.425
ALT	49.05 ± 26.45	46.10 ± 21.43	0.33	0.735
ALT/AST	1.11 ± 0.23	1.05 ± 0.30	1.08	0.279
AST/Platelets	0.55 ± 0.29	0.57 ± 0.27	0.22	0.822
HCV RNA (IU/mL)	949109.96 ± 2361280.34	4383000.0 ± 4878679.35	3.92	< 0.001
Fibrosis F0 F1 F2 F3 F4	 2 (3.4) 17 (29.3) 29 (50.0) 9 (15.5) 1 (1.7)	 0 (0.0) 0 (0.0) 5 (50.0) 5 (50.0) 0 (0.0)	χ^2 8.37	0.079
Fibrosis F0-1 F2 F3-4	 19 (32.7) 29 (50.0) 10 (17.2)	 0 (0.0) 5 (50) 5 (50)	χ^2 7.43	0.024

Data are expressed as mean ± S.D. or number (%).

Zeremski *et al*. in HCV genotype 1-infected population (21).

Although the liver biopsy remains the reference standard for hepatic histologic assessment, the procedure is associated with significant morbidity, sampling bias, and subjectivity that result from both interobserver variability and the use of different scoring systems. Recently, substantial investigation has been conducted to discover parameters, preferably assessed noninvasively, that are capable of distinguishing among different stages of fibrosis. Candidates with promise who were identified individually are subsequently combined into multicomponent indices to increase their discriminatory ability. Currently, 2 noninvasive indices of hepatic fibrosis are commercially available : Fibrosure and Fibrospect. In addition, other noninvasive indices for the detection of hepatic fibrosis, such as APRI (22), Hepascore (23), Fibrometer (24), and Forn's (25), have also been described. Unfortunately, poor ability to identify patients with intermediate stages of fibrosis limits the usefulness of many of these indices (26,27). Novel parameters with improved ability to distinguish among different stages of fibrosis, such as the chemokines described in this report, are required. In addition, maximization of the positive predictive value and negative predictive value should be the aim of future studies.

Although intrahepatic IP-10 levels correlate with necroinflammatory changes and fibrosis in HCV (28), the role of IP-10 in viral clearance is less clear. Low pretreatment IP-10 levels are associated with a rapid decline in HCV viral load during the first 24-48 hours of interferon therapy (29). In this study, we evaluated baseline IP-10 level as a predictive biomarker for treatment response to interferon-ribavirin therapy in hepatitis C genotype 4 infected patients. We demonstrated that low pretreatment serum IP-10 is associated with EVR at week 12 of interferon therapy, HCV RNA negativity at week 24 of therapy and SVR at week 24 post therapy. Higher baseline IP-10 levels were seen in non-responders at weeks 12 and 24 during therapy and relapse at week 24 post therapy. Using pretreatment serum IP-10 (above or below 190 pg/ mL) as a predictive biomarker for treatment response in our cohort revealed a positive predictive value of 86.9% and a negative predictive value of 92.2% for EVR, a positive predictive value of 18.2% and a negative predictive value of 100% for response at week 24 of therapy and a positive predictive value of 25% and a negative predictive value of 91% for SVR. These results are in line with other studies done for hepatitis C genotype 1, confirming that pretreatment IP-10 is lower in patients who subsequently achieve EVR and SVR on therapy compared with nonresponder patients (3,7). The prognostic use of baseline IP-10 levels has also been confirmed in other difficult to treat populations, such as patients coinfected with HCV and human immunodeficiency virus (30), and patients with an elevated viral load and body mass index (31). It is unclear why high IP-10 levels are associated with nonresponse to HCV therapy.

In conclusion, accurate noninvasive assessment of fibrosis is an important yet challenging task that requires the identification and careful evaluation of many parameters and their subsequent grouping into indices to

achieve the highest discriminatory ability. Identification of IP-10 as potential noninvasive fibrosis markers may be an important step in this process. Also pretreatment levels of IP-10 may be useful as first-line tools to identify the majority of HCV genotype 4 patients achieving EVR, HCV RNA negativity at week 24 of therapy and SVR with the currently available therapy. This may be used to stratify patients. Prospective evaluation of baseline serum IP-10 levels in combination with previously validated noninvasive markers of hepatic fibrosis and predictors of EVR and SVR in a larger number of patients will be warranted also, further studies will be needed to show how different is the IP-10 level in HCV infected individuals with no fibrosis from those with fibrosis.

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